Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario

From the Working Group for the Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario, commissioned by the Genetic Testing Advisory Committee.

The Working Group recommends:

1) The application of the herein criteria for the use of genome-wide sequencing for undiagnosed rare genetic diseases in Ontario.

2) That genome-wide sequencing for undiagnosed rare genetic diseases be publically funded in Ontario, based on the herein clinical criteria, testing recommendations and process for the evaluation of longer-term patient outcomes supported by a robust data capture system.

December 2016

Note: This publication is technical in nature and is available in English only due to its limited targeted audience. This publication has been exempted from translation under the French Language Services Act.

Remarque: Cette publication est de nature technique et est disponible en anglais uniquement en raison de son public cible limité. Cette publication a été exemptée de la traduction en vertu de la Loi sur les services en français.
About The Genetic Testing Advisory Committee

New genetic tests are being rapidly developed and promoted as high tech tools to improve diagnostic medicine and facilitate patient treatment. A formal evaluation process is required to assess the validity and effectiveness of new and existing genetic tests to better manage health care spending.

The Genetic Testing Advisory Committee ("GTAC") was established in April 2014 with a three year mandate to review the clinical utility and validity of genetic tests and provide advice to the Ministry of Health and Long-Term Care ("ministry") on the provision of genetic tests in Ontario. The creation of GTAC is in keeping with the direction of the Excellent Care for All Act and the Ontario Action Plan for Health Care to promote the delivery of high-quality health care based on the best available scientific and clinical evidence.

The purpose of GTAC is to review and provide reports on existing and new genetic tests. These reports, in addition to other factors (e.g. implementation feasibility, available funding and resources, system level impacts, and social and ethical factors) will guide the ministry's decision-making on public funding of genetic tests in Ontario.

Review Process

The evaluation process for a genetic test is determined by whether the test is to be provided in Ontario or through the Out-of-Country (OOC) Program Exceptional Access process.

General Stream Process: Ontario Genetic Tests

1. Genetic test selection
2. Evidence-based analysis
   a. Literature review of clinical and academic publications on the genetic test
   b. Referral to an expert sub-committee
   c. GTAC deliberation of the available evidence
3. Draft GTAC report and recommendation prepared
4. Posting draft in Open for Comment
5. Assessment of stakeholder/public comments
6. Posting of finalized GTAC report and recommendations

Exceptional Access Process: OOC Genetic Test

1. OOC genetic test application/submission
2. Assign to appropriate GTAC member for evaluation and recommendation
3. OOC Response to requesting physician.

Website

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1. **Working Group Objectives**

To assist GTAC in the review of the clinical utility and validity of a given genetic test and after a test prioritization process, GTAC convened a Working Group to develop standardized criteria to guide clinicians in the use of genome-wide sequencing for undiagnosed rare genetic diseases in Ontario.

The Working Group was tasked with taking the recommendations outlined in *The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists* (Boycott et al. *J Med Genet* 2015) and adapting them to provide implementation guidance, considering factors such as clinical care/pathway recommendations, diagnostic precision and clinical utility, cost, test sensitivity, test specificity, and the approach to secondary findings.

GTAC would like to thank the Working Group (see Appendix IV) for their diligence and hard work in creating this report.

The following report captures the working group’s recommendations and its contents have been reviewed and approved by GTAC on September 16, 2016.
2. Executive Summary

Genome-wide sequencing, defined as any approach that analyzes and annotates DNA variants on a genome-wide scale, is an appropriate test to facilitate a diagnosis in a patient with a suspected rare disease in certain circumstances. Approaches relevant to the recommendations outlined herein include clinome sequencing (very large panel that includes all genes listed in OMIM and associated with human disease), whole exome sequencing (WES; protein coding portions of the genome), and whole genome sequencing (WGS). Regardless of the approach, clinical genome-wide sequencing, at this time, should be used **to interrogate the genome for nucleotide sequence variants in genes known to cause disease**; variants that are not relevant to the patient's primary indication for diagnostic evaluation, may also be identified. Nucleotide sequence variants in genes not known to be associated with disease will not be reported.

Recommendations include: (1) clinical genome-wide sequencing is an appropriate approach in the diagnostic assessment of a patient for whom there is suspicion of a significant monogenic disease where the baseline evaluation has been completed and the results will directly impact clinical decision-making and care for the individual and/or family being tested; (2) given the complexity of interpretation and counselling, clinical genome-wide sequencing should only be ordered by physicians with sufficient expertise in use of the technology including provision of genetic counselling and ability to obtain informed consent; (3) until the benefits of reporting secondary findings are established, the identification of secondary findings should follow guidelines established by MOHLTC, using a consensus list of medically-actionable genes that is continuously reviewed, updated, and informed by outcome data; (4) clinical sequencing, interpretation, and reporting must be performed in an accredited clinical laboratory and follow the recommendations outlined herein; (5) new diagnoses will be shared with the patient alongside standard-of-care genetic counselling and all patients should be given the option of having coded genome-wide and phenotypic data deposited and stored in an international database to enable genomic discovery; and, (6) this test should be introduced into clinical care alongside a robust evaluative process to optimize the quality and ensure appropriate use of genome-wide sequencing. Finally, these recommendations will be routinely re-evaluated as knowledge of diagnostic and clinical utility of clinical genome-wide sequencing improves.
3. **Detailed Recommendations**

a. **Recommendations for diagnostic assessment**

1.1 Clinical genome-wide sequencing, at this time, should be used to interrogate the genome for nucleotide sequence variants in genes known to cause disease. Clinical WES cannot be used at this time to reliably detect copy number variants or structural variation. Clinical WGS may be used to detect copy number variants and structural variation in addition to sequence variants, though it is not currently a first tier test for such analyses.

1.2 Given the complexity of interpretation and counselling, clinical genome-wide sequencing with open analysis should only be ordered by physicians with sufficient expertise in use of the technology as defined by:
   a. Ability to determine whether clinical genome-wide sequencing is the test of choice for the specific clinical indication, taking into account other genomic available non-genomic tests (such as microarray or metabolic tests) and mutations that will not be detected by this technology (such as trinucleotide repeats); and,
   b. Ability to provide adequate pre-test counseling, including informed consent for primary and secondary findings; and,
   c. Ability to interpret the results of the clinical genome-wide sequencing, including secondary findings, and provide adequate post-test counselling.

1.3 Clinical genome-wide sequencing is considered medically necessary for a phenotypically affected individual when ALL three of the following conditions are met:

   a. Baseline evaluation has been completed (e.g. phenotyping, family history, pretest genetic counselling and consent and, where indicated, chromosome microarray, targeted testing including biochemical testing)

   b. Results will directly impact clinical decision-making and care for the individual and/or family being tested. We assume that all diagnoses will provide an opportunity for anticipatory guidance and enable access to services and support. In addition, results must be anticipated to meet one or more of the following criteria:
   i. Will likely preclude further invasive diagnostic investigations, follow-up, or screening that would be recommended in the absence of testing;
   ii. Provides specific and informed reproductive decision making and family planning; or,
   iii. Will enable identification of at risk family members to identify if they carry the causative mutation and facilitate early intervention or, will enable the ability to rule out risk to family members, thus avoiding long term monitoring.

   c. A genetic etiology is the most likely explanation for the phenotype, supported by a clinical presentation that includes any one of the following:
      i. Moderate to severe developmental or functional impairment;
      ii. Multisystem involvement;
      iii. Progressive clinical course which cannot be explained by another cause; or,
      iv. Differential diagnosis includes two or more conditions (clinical and genetic heterogeneity) that would require evaluation by separate panels and therefore genome-wide sequencing is the more cost-effective approach than individual gene or gene panel testing.
AND the patient does not meet any of the following exclusion criteria:

a. The following clinical indications are NOT an indication for genome-wide sequencing:
   i. Isolated mild intellectual disability or learning disabilities;
   ii. Nonsyndromic autism;
   iii. Isolated neurobehavioural disabilities (e.g. attention deficit disorder); and,
   iv. Isolated neuropsychiatric conditions (e.g. schizophrenia, bipolar disease, Tourette syndrome)

b. Patient’s phenotype is highly specific to a known genetic condition for which optimized genetic testing (including genetic panel testing) exists; in this instance the targeted gene panel should be given priority if it is more sensitive (e.g. Noonan spectrum disorders). In addition, suspected aneuploidy, methylation defects, or trinucleotide repeat disorders would not qualify for clinical genome-wide sequencing.

c. Previous comprehensive panel testing has been completed in the last 3 years (panel contained virtually all known genes for that clinical indication). At this time, clinical genome-wide sequencing is not funded as a reflex test when the appropriate panel has not yielded a molecular diagnosis. If a panel did not reveal a diagnosis in the past 3 years but there is a strong argument to be made for clinical genome-wide sequencing, this application would be reviewed by an expert panel and justification must be made for the test indication.

d. Baseline evaluation (physical examination, investigations) has not been completed for the clinical indication [see 1.3 (a)].

e. A likely non-genetic etiology has been identified to explain the symptoms (e.g. environmental exposures, injury, or infection).

1.4 Testing should always include the affected individual. In discussion with the clinical laboratory, additional affected relatives or unaffected parents, if available, may be analyzed concurrently to facilitate and improve variant interpretation. We suggest the following approaches:
   o Trios are the preferred strategy for WES for undiagnosed patients with no family history of similarly affected individuals.
   o In the context of consanguinity, a singleton approach is preferred.
   o In the context of presumed recessive inheritance, one affected individual and an unaffected parent OR two affected individuals is the preferred approach.
   o In the context of presumed dominant inheritance, the analysis of additional affected individuals may significantly reduce the number of potentially deleterious heterozygous variants; concurrent testing of the most distantly related and available affected family member should therefore be considered.
   o If X-linked inheritance is clear, a singleton approach with filtering for X-linked variants is preferred.
   o If a mitochondrial genome mutation is suspected, the mitochondrial genome should be analyzed directly using alternate and more sensitive approaches.

1.5 Limited evidence currently exists supporting the use of genome-wide sequencing in the prenatal setting. Specific guidelines for pre-natal indications must therefore be developed when sufficient evidence has become available. In the interim, requests for clinical genome-wide sequencing in this setting would require evaluation for appropriateness by the Exceptional Access process.

1.6 Post-mortem genome-wide sequencing may be indicated if the affected proband meets the inclusion/exclusion criteria outlined in this document, regardless of age of death (prenatal, pediatric, or adult).
b. Pre-test recommendations

2.1 Standard clinical assessment, including detailed phenotyping, must be undertaken and documented prior to testing to facilitate interpretation of the genome-wide variants. Phenotyping should be documented using the standardized nomenclature of Human Phenotype Ontology. Such documentation must be forwarded to the laboratory in the format preferred by the lab (laboratory specific requisition and/or software such as Phenotips (https://phenotips.org; Girdea, 2013, etc.).

2.2 Prior to testing, genetic counselling for the patient/family must be undertaken and documented in the medical record by a qualified health care provider with a thorough understanding of clinical genome-wide sequencing, including issues unique to both pediatric and adult patients. Informed consent should be formally documented prior to testing, utilizing the program specific consent form. This consent form should become part of the patient medical record.

2.3 Documentation of informed consent and preferences regarding secondary findings must be documented on the laboratory requisition.

2.4 The counselling and consent process should include but not be limited to the following items:
   - Brief description of the test and the rationale for offering, tailored to the information needs of the patient/family;
   - Information regarding the limitations of the test methodology;
   - Information regarding the possibility of secondary findings.
   - Discussion of expected outcomes and what will, and will not, be reported from the test, including:
     - variant classes (i.e. pathogenic, VUS, benign) related to genes relevant to the primary clinical findings;
     - Secondary findings and the choices pertaining thereto with documentation of patient preferences;
   - Possible (or definite) need for unaffected parental or family member samples and the type of analysis required; clinical genome-wide analysis or follow-up Sanger testing. Unaffected family members should be informed that if their sample is to undergo genome-wide analysis it is only to interpret the clinical significance of the variants in the proband potentially related to the clinical indication. Independent reports will not be issued for unaffected family members and analysis for secondary findings will not be undertaken. Any follow-up of secondary findings identified in the proband and possible impact on family members represent distinct healthcare interactions;
   - Potential implications of test results on issues such as medical care, possible insurance and discrimination, impact for other family members, recommendations for cascade testing for family members, etc;
   - An explanation of what will happen with data, including how long they will be stored as per the guidelines of the laboratory;
   - Potential for reinterpretation of variants or re-analysis of data for the primary indication (should a diagnosis remain lacking) and how this will occur. This would involve a new requisition and consent process at the request of the health-care provider.
   - Consent for clinical testing should follow standard guidelines and include sufficient time to understand and decide whether or not to proceed with genome-wide testing.

2.5 All patients/families should be aware that they must opt out of having anonymized genotypic and high-level phenotypic data deposited and stored in an international database to assist in interpretation of disease-causing variants.
2.6 All patients/families should be given the option of having coded genome-wide and phenotypic data deposited and stored in an international database to enable genomic discovery. The release of genome-wide sequencing data for secondary use would require a distinct consent that would waive laboratories of responsibility beyond what they have included in their clinical report. This consent would be obtained and provided by the referring clinician/institution.

2.7 All patients/families should be given the opportunity to enroll in current or future research studies to understand the relationship of genome-wide variants found in them and disease pathogenesis. The release of patient materials for functional studies would require a distinct consent that would be obtained and provided by the referring clinician/institution.
c. Recommendations for secondary findings

The recommendations set out herein refer to analysis of genome-wide sequencing data that includes all clinically relevant genes (e.g. OMIM, Orphanet, other curated set), as defined by the laboratory. At present, the reporting of secondary findings (also referred to as incidental findings, additional findings) remains a controversial issue. This is primarily due to the lack of empiric knowledge regarding the downstream psychosocial and clinical impact (i.e. potential benefits and risks) of gaining such knowledge for patients/families and on the health care system as well as difficulty in interpreting these variants in the absence of personal and family history of potentially related signs and symptoms. Consequently, until the benefits of reporting secondary findings are established, a cautious approach is recommended.

3.1 The MOHLTC should support the development of a working group that will develop and maintain a consensus list of medically-actionable genes to be reported by Ontario-based laboratories. This review of this list will be informed by data collected and analyzed as part of Section f (6.4).

3.2 Only pathogenic and likely pathogenic variants in genes from this consensus list will be reported for secondary findings in the proband. Variants of uncertain significance WILL NOT be reported. Genome-wide data from unaffected family members generated for the purpose of interpreting variants identified in the proband will NOT be analyzed for secondary findings. Carrier status for conditions unrelated to the primary test indication WILL NOT be reported.

3.3 Follow-up of secondary findings in the proband and at risk family members would represent a distinct healthcare interaction.

3.4 A process for obtaining informed consent and for communicating potential secondary findings must be in place (see Section b). The return of secondary findings, and the options pertaining thereto are as follows:
   a. Competent affected individuals should be given the option prior to testing to receive (or not receive) secondary findings unrelated to the primary test indication.
   b. In children deemed not competent to participate in meaningful decision making (i.e. too young or intellectually disabled), secondary results that reveal risk for a highly-penetrant condition that is medically-actionable during childhood should be reported to the parents. A child’s risk for medically-actionable adult-onset genetic conditions should not be communicated unless: (1) the parents request such disclosure; AND, (2) disclosure of the information could prevent serious harm to the health of a parent or family member, as determined on a case-by-case basis. There is no obligation to re-contact pediatric patients as adults to let them know of potentially later onset monogenic diseases. However, parents/guardians should be encouraged to share test results with their children at appropriate time points.
   c. For incompetent adults, results revealing a highly-penetrant medically-actionable condition should be reported to the legal representative, unless the incompetent adult explicitly refused to receive such actionable results while still competent.

3.5 Prior to disclosure of medically actionable secondary variants, patients/families should be given the opportunity to affirm or deny their previous decision to learn of secondary findings. Disclosure of secondary variants should be staged according to the wishes of the patient/family (i.e. at appointment subsequent to discussion of variants related to primary findings); however, some discretion must be undertaken by the clinician based on the potential urgency of the secondary variant.
d. Clinical testing and results reporting recommendations

4.1 Clinical sequencing, interpretation and reporting must be performed in a clinical laboratory licensed within the appropriate jurisdiction to perform these tests. Licensing and accreditation details should be included on the report or readily available from the laboratory website.

4.2 Requests for sequencing must be accompanied by relevant family history, detailed phenotypic information on the patient, in standardized HPO terms (collected by the laboratory requisition or software such as PhenoTips), and details of any relevant previous genetic testing.

4.3 Laboratories reporting results based on genome-wide sequencing must include specific information describing the sequencing methodology used (including test sensitivity, reagent kit version numbers, instrumentation used), the analytical pipeline (including its limitations), and the approach to analysis. Supplemental information should include a reference to the list of genes examined, and any relevant regions routinely not covered by the sequencing methodology that have not been addressed by Sanger sequencing.

4.4 Patient specific coverage for a particular gene, or set of genes, must be available upon request.

4.5 All reports should clearly state the test description; reason for referral; result (Positive Negative, Inconclusive); DNA findings; interpretations; recommendation; background information (in relation to the reason for referral and for other DNA findings included on the report); test methodologies; and, references. Background information should include mode of inheritance, carrier status, risk to other family members, disease presentation/phenotype, as appropriate.

4.6 DNA findings above should include, at minimum, the cDNA position and nucleotide change (according to HGVS nomenclature) with the corresponding reference sequence version (NM number); the amino acid change; the classification of the DNA variant (according to published guidelines, such as ACMG 2015); and, the zygosity. The gDNA position and nucleotide change with the corresponding genome reference or build is optional. The significance of alternative transcripts with multiple transcript designations must also be considered.

4.7 The laboratory report for clinical genome sequencing must include an interpretation by a clinically trained and certified PhD or MD Molecular Geneticist; in Canada, this would typically be one with CCMG or ABMG certification. When appropriate, consultation with the referring clinician on variant interpretation in the context of the clinical presentation is strongly advised.

4.8 Interpretation of results should include assessment of current peer-reviewed literature, predictive software and databases of known variation, and take into account the known limitations of these resources. Interpretation should follow standard published guidelines (Lerner-Ellis, 2015).

4.9 In instances where unaffected familial samples (i.e., parents in trio analysis) are analyzed to help interpret the patient’s sequencing data, separate reports will not be generated and samples will not be analyzed individually for secondary findings. Evidence of misassigned paternity, incest, etc., should be dealt with following current principles and institutional policies.

4.10 Secondary findings, if present, should be in a separate section from the primary findings and indicate the reporting guidelines being followed.
4.11 Release of patient genome-wide data for further analysis is case specific and such secondary use of data will require a distinct research consent and the lab will not release data unless there is documentation of this consent and a SOP in place for data transfer.

4.12 The MOHLTC should enable a process for accreditation and external quality assurance of Ontario labs conducting genome-wide sequencing tests. This includes, but is not limited to, setting standards for, and ongoing evaluation of, technical performance characteristics, analytical quality, and reporting of results.
e. Post-test recommendations

5.1 The ordering clinician should review the report and place the findings into context with other relevant medical considerations when discussing the results with the patient/family.

5.2 The patient (and family when appropriate) must receive standard-of-care genetic counselling and management regarding any new diagnosis.

5.3 The laboratory must provide access to expert consultation on the interpretation of a variant if requested by the referring clinician.

5.4 The laboratory may suggest that additional laboratory-based diagnostic follow up is required to verify the genome-wide sequencing findings (including a targeted test; CNV test or methylation specific test, inclusion of parents or other unaffected or affected family members).

5.5 In the case in which the laboratory identifies multiple candidate variants, additional literature review, database searching, and phenotyping should be considered by the ordering clinician.

5.6 If no pathogenic variant is identified, patients should be counselled that:
   o DNA variant may still be present that was not detectable using the sequencing method.
   o Further analysis might lead to a diagnosis at a later date when more knowledge is available. This may involve re-testing rather than re-analysis, at the discretion of the laboratory. The WG recommends that the ministry establish criteria for both the frequency and funding for this re-analysis. Requests for re-analysis of the sequencing data should be initiated by a referring physician. Two types of re-analysis may occur: 1) a single-gene analysis motivated by a novel gene discovery associated with the phenotype or a new phenotypic finding that broadens the differential diagnosis; or, 2) a full re-analysis. The decision to re-analyze must be based on expansion of genetic knowledge specific for the disease (pertinent to phenotype), time since last analysis, clinical presentation, and management implications for the patient and family.
   o Further analysis of the sequencing data through research may be an option. A clear distinction should be made between clinical and research analysis, and explicit informed consent obtained for the latter, including that research test results would need to be confirmed in an approved laboratory prior to being used to guide medical management. If further research analysis is initiated the clinical laboratory will release the appropriate data at the direction of the patient; the data might be released to the patient or a 3rd party explicitly delegated by the patient (clinician, researcher, family member, etc.).

5.7 The laboratory will retain patient data (VCF file) for a period of at least 3 years to allow reanalysis or research investigation.
f. **Outcome measures**

6.1 To optimize the quality and ensure appropriate use of genome-wide sequencing, it is essential to introduce this test into clinical care alongside a robust evaluative process. The proposed evaluation is characterized as a service-oriented quality improvement process.

6.2 Resources required to establish and sustain an outcomes-oriented data infrastructure should be provided by MOHLTC. Opportunities for MOHLTC to partner with key stakeholders (e.g. Ontario Genomics Institute, Genome Canada, Health Quality Ontario) to sustain and expand this infrastructure should be actively sought.

6.3 The proposed evaluation process is framework-driven and is informed by the ‘chain of evidence’ concept, which assists with connecting the use of the test to clinically important health or healthcare-related outcomes (Appendix II). Fryback and Thornbury’s hierarchical model best articulates appropriate intermediate outcomes to evaluate genome-wide sequencing by including ‘diagnostic thinking efficacy’ and ‘therapeutic efficacy’ as core domains.

6.4 To capture these outcomes, a user-friendly data collection tool should be developed. The Efficacy Form (Appendix I) serves as an example of a data collection tool that could be used to support the proposed evaluation. **Part A** would be completed at the time that clinical genome-wide sequencing is ordered and asks ordering clinicians to indicate what their diagnostic care plan for the index case would be if sequencing was not available. **Part B** would be completed following the disclosure of sequencing results to the index family and asks clinicians to indicate: (a) what type of sequencing result was identified in the index case (i.e. Diagnosis, No Diagnosis, Partial Diagnosis, and/or Secondary Finding); (b) whether the sequencing result influenced diagnostic thinking; and, (c) how the clinical care plan was guided by sequencing result(s). **Part A** of this form would take <2 minutes to complete and **Part B** would take <5 minutes to complete; clinicians should be incentivized to complete these forms.

6.5 The data collection tool should be centralized, electronic, and mandatory. All clinical settings that participate in clinical genome-wide sequencing should have access to a shared external data capture portal and should enter outcomes-oriented data (defined by the data collection tool) on an ongoing basis. Interim analyses should be conducted (annually) to recalibrate the provincial genomic sequencing strategy to optimize its delivery.

6.6 This process and structure developed herein should be sustained and leveraged for future provincial/national Precision Medicine initiatives.
Appendix I. Forms

Section a: Recommendations for Diagnostic Assessment

Form 1: Patient Criteria for Genome-Wide Sequencing

Section f: Outcome Measure

Form 2: Efficacy Form
Appendix II. Framework for Evaluating the Introduction of Genome-Wide Sequencing in Ontario

A. The Evaluation Framework

A number of evaluation frameworks have been used for synthesizing the published evidence about the accuracy and utility of genetic tests. Some of these include: the Evaluation of Genomic Applications in Practice and Prevention frameworks (EGAPP), the Analytic validity; Clinical validity; Clinical utility; and Ethical, legal, and social implications framework (ACCE model), the Fryback-Thornbury model, and the USPSTF framework for screening. All four frameworks cover three common domains of evaluation: analytic validity, clinical validity, and clinical utility of the test. The ACCE framework and the Fryback-Thornbury model extend to dimensions that reflect upon cost and societal impact of the test (Figure 1: A comparison of key evaluation frameworks for clinical tests). Unlike the other models, the Fryback-Thornbury model also attends to the notions of diagnostic thinking and therapeutic efficacy.

Figure 1. A comparison of key evaluation frameworks for clinical tests

Note: This figure was created by ECRI Institute based on the specified evaluation frameworks. For a detailed description of each included framework, refer to the original references.15,32,34-41

<table>
<thead>
<tr>
<th>Domain 1: Analytical validity</th>
<th>Domain 2: Clinical validity</th>
<th>Domain 3: Clinical utility</th>
<th>Domain 4: Ethical, legal, and societal implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCE = ACCE initiative (ACCE stands for analytic validity, clinical validity, clinical utility, and ethical, legal, and social implications); EGAPP = Evaluation of Genomic Applications in Practice and Prevention initiative; USPSTF = U.S. Preventive Services Task Force</td>
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Recommendations for the Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario
B. Levels of Efficacy

<table>
<thead>
<tr>
<th>Level 1: Technical Efficacy (i.e. analytic validity)</th>
<th>Level 2: Diagnostic Accuracy Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does the test measure what it purports to measure?</td>
<td>What are the medical test characteristics of the test (e.g., sensitivity, specificity)?</td>
</tr>
</tbody>
</table>

**Level 1: Technical Efficacy**

In the laboratory setting, does the test measure what it purports to measure? (e.g., the presence of a gene mutation). Analytic validity is a function of many factors, such as analytic accuracy, precision, analytic sensitivity and specificity, reportable range of test results for the test system, reference range, or normal values. These performance parameters will be evaluated by participating laboratories in accordance with standard laboratory procedures.

**Level 2: Diagnostic Efficacy**

Does the test result distinguish patients with and without the target disorder among patients in whom it is clinically reasonable to suspect that the disease is present?

**Level 3: Diagnostic Thinking Efficacy**

Does the medical test help clinicians come to a diagnosis?

**Level 4: Therapeutic Efficacy**

Does the medical test aid in planning treatment?

**Level 5: Patient Outcome Efficacy**

Do patients benefit from the use of the test?

**Level 6: Societal Efficacy**

Cost–benefit and cost-effectiveness

- Level 1: Technical Efficacy (i.e. analytic validity) refers to the ability of genome-wide sequencing to accurately and reliably measure the characteristic it is intended to measure (e.g., the presence of a gene mutation). Analytic validity is a function of many factors, such as analytic accuracy, precision, analytic sensitivity and specificity, reportable range of test results for the test system, reference range, or normal values. These performance parameters will be evaluated by participating laboratories in accordance with standard laboratory procedures.

- Level 2: Diagnostic Efficacy (i.e. clinical validity) refers to the ability of CGES to predict the presence of absence of a clinical condition or predisposition. This performance parameter will be monitored by participating laboratories in accordance with standard laboratory procedures. As per published literature, a diagnostic rate of 30% will be applied as our provincial benchmark (Yang, 2014; Yang, 2015; Lee, 2014; Soden, 2014).

- Levels 3: Diagnostic Thinking Efficacy. In the clinical context, patients’ health outcomes cannot be affected by a genetic test result unless the attending clinician is led to do something different than would have been done without the information provided by the test (i.e. decisional impact). Similarly, the clinician’s choice of management should not change unless something has changed in the diagnostic thinking, all other things equal. As an alternative to decisional impact, genetic test result may have non-decisional but still important impact by changing the differential diagnosis, strengthening an existing hypothesis, or reassuring the clinician that a speculated diagnosis has or has not been confirmed. An empiric approach to ascertaining diagnostic thinking efficacy for WES is presented in the Efficacy Form (Appendix I). Benchmarks cannot be predetermined. This process will assist in defining benchmarks going forward.

- Level 4: Therapeutic efficacy (i.e. clinical utility). A genetic test result may influence the clinician’s diagnostic thinking and subsequently, have an impact on the patient’s clinical trajectory of care. The intent of the care plan will typically be tied to the type of genetic test result. When a diagnostic variant has been identified, care plans may be tailored to suit prognoses that are better defined (e.g. sub-specialist referrals, imaging or surveillance plans, medication implications, family member testing, reproductive planning). When no variant or a variant of uncertain diagnostic significance has been identified, care plans may be tailored towards more extensive diagnostic investigations (e.g. muscle biopsies, additional genetic analyses, family member testing). For the purpose of the proposed evaluation process, a pre/post test design will enable a description of care plans triggered by WES compared to standard care (Efficacy Form). Benchmarks cannot be predetermined. This process will assist in defining benchmarks going forward.
• **Levels 5: Patient Outcome Efficacy** 'Improved health' can be defined and measured in many different ways (e.g. reduced morbidity, increased life expectancy, improved quality of life, reduced pain, reduced number of invasive procedures). Patient outcomes research enables a determination of the ways in which the expected cost of an intervention (i.e. genetic test) can be weighted against expected benefits (e.g. improved quality of life, reduced number of investigations) and as such, can be used as a rational guide for payers, clinicians, and patients to decide about whether or not to reimburse, order, or pursue (respectively) a particular intervention (i.e. genetic test) in the first place. Patient outcomes research is out of scope for the purpose of the proposed WES evaluation, but the data infrastructure that is created should be forward thinking and flexible in order to accommodate such research in the near future. Benchmarks: N/A at this time.

• **Level 6: Societal Efficacy.** The question of societal efficacy goes beyond the question of individual risks and benefits. It poses questions of acceptability and resource allocation. It asks whether the cost for the use of a given genetic test is acceptable – to society as a whole - even though individual patients may benefit. Questions of benefit-risk trade-offs, ethical considerations, and resource allocation feature herein. While societal efficacy will be an implicit consideration over the course of the proposed WES evaluation process, fulsome consideration of this element will occur at a later stage. Benchmarks: N/A at this time.

**D. Limitations**

- The trajectory of care that is captured by this process will reflect a projected trajectory; it will not reflect actual resources consumed (i.e. health care dollars spent).
- The trajectory of care that is captured will reflect upon a prospective window of 6-12 months as this is the characteristic follow-up period for children who receive routine care from tertiary care genetics centres. It will not capture recommended services for the longer term.
- Health/functional outcomes will not be captured by this tool; only intermediate outcomes related to diagnostic and therapeutic efficacy will be captured at this point in time.

**E. Deliverables**

- A conceptual model tailored to the evaluation of genome diagnostics.
- A centralized, data capture system and data collection protocol suited to long-term evaluation and ensuring quality of genome diagnostics in Ontario. This process and structure should be sustained and leveraged for future provincial/national Precision Medicine initiatives.
- Preliminary evidence that reflects upon the technical, diagnostic and therapeutic efficacy of genome-wide sequencing as a diagnostic tool for rare genetic disorders and will be used to inform the implementation of clinical genome-wide sequencing in Ontario.
- A team of laboratory scientists, clinicians, and evaluation scientists well-positioned to expand the proposed evaluation concept and design to other provincial and national centres for the purpose of ongoing quality improvement and research.
Appendix III. References


Appendix IV. Working Group Membership

Dr. Kym Boycott, Children’s Hospital of Eastern Ontario (Chair)
Dr. Sarah Bowdin, Hospital for Sick Children
Dr. Dennis Bulman, Children’s Hospital of Eastern Ontario
Dr. Pranesh Chakraborty, Children’s Hospital of Eastern Ontario
Dr. Jordan Lerner-Ellis, Mt Sinai Hospital
Dr. Robin Hayeems, Hospital for Sick Children
Dr. Sharan Goobie, London Health Sciences Centre
Dr. Peter Ray, Hospital for Sick Children
Ms. Cheryl Shuman, Hospital for Sick Children

Note: This publication is technical in nature and is available in English only due to its limited targeted audience. This publication has been exempted from translation under the French Language Services Act.

Remarque: Cette publication est de nature technique et est disponible en anglais uniquement en raison de son public cible limité. Cette publication a été exemptée de la traduction en vertu de la Loi sur les services en français.
MOHLTC Process for Approval of Whole-Exome Sequencing

Background:
Whole-exome sequencing (WES) is a useful diagnostic test in a number of clinical scenarios for patients with known or suspected genetic disease. The Genetic Testing Advisory Committee (GTAC) to the Ontario Ministry of Health and Long-term Care was tasked with taking the recommendations outlined in *The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists* (Boycott et al. J Med Genet 2015) and providing implementation guidance for the province of Ontario, considering factors such as diagnostic precision, cost, test sensitivity, test specificity, and ultimate impact on health outcomes. Guidelines were developed on the basis of evidence to facilitate clinical translation of this technology and contribute to best practices in Ontario. The clinical indications we outline here will evolve over time as more data are generated and at this time the following criteria for approval should be considered *provisional*. Data will be requested regarding outcomes of the test for your patient and your cooperation in providing this information will contribute to these evolving best practices.

Approval Process:
- The accompanying form was developed to reflect the developed guidelines for approval of clinical WES. This form must be completed and accompany the application to the MOHTLC.
- If the OOC health services are received without written prior approval from the ministry, then the services are not eligible for reimbursement and all costs associated with these services will be the sole responsibility of the patient.
- If the approval criteria are not met for a particular patient, the case will be reviewed by the Exceptional Access Committee of GTAC and additional information will be requested for this deliberation.
- At this time WES is NOT funded as a reflex test when the appropriate genetic panel has not yielded a molecular diagnosis. If a panel test was negative in the past 3 years but there is a strong argument to be made for WES at this time, please attach a letter justifying the need for WES and this will be reviewed by the Exceptional Access Committee of GTAC.

Laboratory and Analysis Approach:
Testing should always be done on the affected individual.

Additional affected relatives or unaffected parents, if available, may be analyzed concurrently to facilitate and improve variant interpretation. We suggest the following approaches:
- Trios are the preferred strategy for WES for undiagnosed patients with no family history of similarly affected individuals.
- In the context of consanguinity, a singleton approach is preferred.
- In the context of recessive inheritance, one affected individual and an unaffected parent OR two affected individuals is the preferred approach.
- If X-linked inheritance is clear, a singleton approach with filtering for X-linked variants is preferred.
- If a mitochondrial genome mutation is suspected, the mitochondrial genome should be analyzed directly using alternate and more sensitive approaches.

Options if WES is Unrevealing:
If the clinical presentation of your patient is not explained after clinical WES you can consider the following:
- Request re-analysis of the data in 1 years time.
- With appropriate consent, share your patient’s phenotypic data, candidate gene and/or WES data with one of the many global sharing initiatives for unsolved rare diseases (e.g. PhenomeCentral; [www.phenomecentral.org](http://www.phenomecentral.org)).

At this time WGS is NOT funded by the MOHLTC as either a primary or reflex test.
Please complete this eligibility form and the pre-test form and send it along with your application for OOC funding requesting clinical WES.

RE: patient Information sticker here

Please confirm that clinical WES is indicated and appropriate for your patient.

Patient must meet one or more from Clinical Presentation AND Management Impact (check all that apply)

Clinical Presentation:
- □ Moderate to severe developmental or functional impairment
- □ Multisystem involvement
- □ Progressive clinical course
- □ Differential diagnosis includes two or more conditions that would be evaluated in separate panels

Management Impact:
- □ Will limit further invasive diagnostic investigations
- □ Results allow for specific and informed reproductive decision making
- □ Will enable identification of at risk family members and facilitate early intervention

AND

☐ I confirm that:
  - o Detailed phenotypic characterization (physical examination, investigations) has occurred and is documented.
  - o Pretest genetic counseling and consent has been completed.
  - o Chromosomal microarray has been completed and does NOT explain the patient’s phenotype (for patients with developmental delay, intellectual disability, multiple congenital anomalies, dysmorphic features).
  - o Other causative circumstances (e.g. environmental exposures, injury, and infection) do NOT explain the patient’s clinical presentation.
  - o Previous targeted testing unrevealing (*if specific monogenic disorder suspected).
  - o Previous comprehensive panel testing has NOT been in completed in the last 3 years (panel contained virtually all known genes for that clinical indication).

☐ I confirm that the patient does NOT have:
  - o Isolated mild intellectual disability or learning disabilities;
  - o Nonsyndromic autism;
  - o Isolated neurobehavioural disabilities (e.g. attention deficit disorder).
  - o A phenotype highly specific to a known genetic condition for which optimized genetic panel testing exists.
    If so, then the targeted gene panel should be given priority assuming it is more sensitive (e.g. Noonan spectrum disorders).
I am requesting:

☐ Singleton and rationale

☐ Trio and rationale

I confirm that I am a physician in Ontario practicing in Medical Genetics with:

1. Expertise in performing a clinical genetics evaluation including family history, genetic-focused medical history and physical examination, and have a critical understanding of prior genetic evaluations.
2. Expertise in determining whether clinical WES is the test of choice for the specific clinical indication, prioritizing other available tests as appropriate.
3. Expertise in providing adequate pre-test counseling, including informed consent for primary and incidental findings.
4. Ability to interpret the results of the clinical WES and provide adequate post-test counseling.

I confirm that I will participate in the evaluation strategy of clinical WES for my patient by completing the outcomes data collection form at the time the results are shared with my patient/family.

Physician Signature: ________________________ Date: ________________________

CSN #: ____ ____ ____ ____ ____
Part A: Complete at time of WES request

<table>
<thead>
<tr>
<th>Q1. Patient’s diagnostic care plan</th>
<th>Check all tests that have been performed</th>
<th>Check all tests that you would request if WES was not available at this point</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory tests:</strong></td>
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<tr>
<td>Cytogenetic testing (specify):</td>
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<td>Molecular testing (specify):</td>
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<tr>
<td>Metabolic testing (specify):</td>
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<tr>
<td><strong>Medical imaging:</strong></td>
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<tr>
<td>MRS (specify):</td>
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<td>MRI (specify):</td>
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<td>CT (specify):</td>
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<td>U/S (specify):</td>
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<td>X-Ray (specify):</td>
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<td>Skeletal Survey:</td>
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<td>Bone Age:</td>
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<td>Echo:</td>
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<tr>
<td>Other (specify):</td>
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<tr>
<td><strong>Invasive procedures:</strong></td>
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<tr>
<td>Skin biopsy</td>
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<td>Muscle biopsy</td>
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<td>Nerve biopsy</td>
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<td>Other (specify):</td>
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<td><strong>Other:</strong></td>
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</tbody>
</table>
PART B: Post-WES care plan

<table>
<thead>
<tr>
<th>Q1. WES result (check all that apply)</th>
<th>Diagnosis</th>
<th>No diagnosis</th>
<th>Partial Diagnosis</th>
<th>Secondary Finding</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Q2. Diagnostic thinking efficacy <em>(Check all that apply)</em></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>WES result helped to establish a diagnosis</td>
<td></td>
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<tr>
<td>WES result helped to establish prognosis</td>
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<tr>
<td>WES identified new risks for the patient</td>
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<tr>
<td>WES halted the diagnostic odyssey</td>
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<tr>
<td>WES altered differential diagnosis</td>
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<tr>
<td>WES result ruled out potential diagnoses (that were previously hypothesized to be the cause)</td>
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<td>WES result indicated that further dx investigations were required</td>
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<tr>
<td>Other:</td>
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</tbody>
</table>
Q3. WES result altered the patient’s care plan by prompting (select all that apply):  

<table>
<thead>
<tr>
<th>Sub-specialist referral(s)</th>
<th>Primary Diagnosis</th>
<th>Secondary Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy/Immunology</td>
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<tr>
<td>Cardiology</td>
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<tr>
<td>Chronic Pain</td>
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<tr>
<td>Dentistry</td>
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<td>Dermatology</td>
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<td>Developmental Peds</td>
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<td>Endocrinology</td>
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<td>ENT</td>
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<td>Gastroenterology</td>
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<td>General Surgery</td>
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<td>Gynecology</td>
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<td>Hematology</td>
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<td>Metabolic</td>
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<td>Nephrology</td>
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<td>Neurology</td>
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<td>Neurosurgery</td>
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<td>Oncology</td>
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<tr>
<td>Ophthalmology</td>
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<tr>
<td>Orthopedics</td>
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<tr>
<td>Pharmacology</td>
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<tr>
<td>Physical Medicine &amp; Rehab</td>
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<td>Plastics</td>
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<tr>
<td>Psychiatry/Addiction Medicine</td>
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<td>Respirology</td>
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<td>Rheumatology</td>
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<td>Sports Medicine</td>
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<td>Thoracic Surgery</td>
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<tr>
<td>Urology</td>
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<tr>
<td>Vascular Surgery</td>
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<td>Other:</td>
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**Allied health referrals**

<table>
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<tr>
<th>Allied health referrals</th>
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<tbody>
<tr>
<td>Audiology</td>
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<tr>
<td>Dietetics/Nutrition</td>
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<td>Occupational Therapy</td>
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<tr>
<td>Physiotherapy</td>
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<tr>
<td>Q3. WES result altered the patient’s care plan by prompting (select all that apply):</td>
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<td>-------------------------------------------------</td>
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<tr>
<td>Speech Language Therapy</td>
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<tr>
<td>Genetic Counselling</td>
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<tr>
<td>Psychology</td>
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<td>Other:</td>
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</table>

**Medical imaging**

| MRS:                                             |                 |                 |
| MRI (specify):                                   |                 |                 |
| CT (specify):                                    |                 |                 |
| U/S (specify):                                   |                 |                 |
| X-Ray (specify):                                 |                 |                 |
| Skeletal survey:                                 |                 |                 |
| Bone age:                                        |                 |                 |
| Echo:                                            |                 |                 |
| Other:                                          |                 |                 |

**Laboratory investigations**

| Genetic (specify):                               |                 |                 |
| Biochemical (specify):                           |                 |                 |
| Cytogenetic (specify):                           |                 |                 |
| Other:                                          |                 |                 |

**Medication implications**

| A new medication                                 |                 |                 |
| A medication change (i.e. stoppage, dosage change) |                 |                 |
| Identification of a medication sensitivity       |                 |                 |
| Other:                                          |                 |                 |

**Procedures (specify):**

**Discontinuation of specific health services (specify):**

**Genetic counselling for family members**

| related to recurrence risk                       |                 |
| related to cascade family testing               |                 |
| related to prenatal testing options             |                 |
| related to prenatal care                        |                 |

**Research opportunities for the patient/family (specify):**

**Other**